A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP3655.

Early-Life Cadmium Exposure and Bone-Related Biomarkers: A Longitudinal Study in Children

Annachiara Malin Igra,[1] Marie Vahter,[1] Rubhana Raqib,[2] and Maria Kippler[1]

[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[2]Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladeshs

Background: Chronic cadmium exposure has been associated with osteotoxicity in adults, but little is known concerning its effects on early growth, which has been shown to be impaired by cadmium.

Objectives: Our objective was to assess the impact of early-life cadmium exposure on bone-related biomarkers and anthropometry at 9 y of age.

Methods: For 504 children in a mother–child cohort in Bangladesh, cadmium exposure was assessed by concentrations in urine (U-Cd, long-term exposure) and erythrocytes (Ery-Cd, ongoing exposure) at 9 and 4.5 y of age, and in their mothers during pregnancy. Biomarkers of bone remodeling [urinary deoxypyridinoline (DPD), urinary calcium, plasma parathyroid hormone, osteocalcin, vitamin D3, insulin-like growth factor (IGF) 1, IGF binding protein 3, thyroid stimulating hormone] were measured at 9 y of age.

Results: In multivariable-adjusted linear models, a doubling of concurrent U-Cd was associated with a mean increase in osteocalcin of 2.7 ng/mL (95% CI: 0.042, 5.9) and in urinary DPD of 22 nmol/L (95% CI: 12, 32). In a combined exposure model, a doubling of maternal Ery-Cd was associated with a mean increase in urinary DPD of 15 nmol/L (95% CI: −0.047, 30). Stratifying the osteocalcin model by gender ($p_{interaction} = 0.001$), a doubling of concurrent U-Cd was associated with a mean decrease in osteocalcin of −4.3 ng/mL (95% CI: −8.5, −0.080) in boys and a mean increase of 9.4 ng/mL (95% CI: 5.4, 13) in girls. The same pattern was seen with U-Cd at 4.5 y of age ($p_{interaction} = 0.016$). Children's U-Cd and Ery-Cd, concurrent and at 4.5 y of age, were inversely associated with vitamin D3.

Conclusions: Childhood cadmium exposure was associated with several bone-related biomarkers and some of the associations differed by gender. https://doi.org/10.1289/EHP3655

Introduction

Environmental exposure to the toxic metal cadmium occurs primarily via food, especially cereals and root vegetables, and smokers are additionally exposed via elevated cadmium concentrations in tobacco (EFSA 2009). In adults, primarily in the upper-middle-aged to elderly, chronic low-level cadmium exposure has, besides the well-known kidney effects, been associated with decreased bone mineral density and increased risk of osteoporosis and fractures (Åkesson et al. 2014). The cadmium-related bone effects were previously considered to be secondary to kidney damage, especially at high cadmium exposure (Jin et al. 2004). More recent observational studies have, however, found cadmium exposure to be associated with adverse effects on bone, even in the absence of renal tubular dysfunction (Nawrot et al. 2010; Schutte et al. 2008), which is supported by experimental studies (Bhattacharyya 2009).

How cadmium affects bone in children, who might be particularly susceptible during rapid growth and bone accrual, is not clear. On the one hand, it is becoming increasingly evident that cadmium may affect children's growth because prenatal exposure to cadmium has been associated with smaller size at birth (Johnston et al. 2014; Kippler et al. 2012; Sun et al. 2014) as well as with height (Tian et al. 2009) and weight at preschool age (Lin et al. 2011). In addition, childhood cadmium exposure has been inversely associated with height, weight, and growth velocity at 5 y of age (Gardner et al. 2013). On the other hand, a couple of cross-sectional studies have assessed the association between cadmium exposure and different bone-related biomarkers, with conflicting results (Sughis et al. 2011; Yang et al. 2013). In Pakistani children 8–12 y of age ($n = 155$), urinary cadmium (U-Cd) ($\sim$0.18–1.8 μg/L) was positively associated with urinary DPD and urinary calcium (Sughis et al. 2011), suggesting increased bone resorption. However, no association was observed between the blood cadmium concentrations (range 0.36–1.45 μg/L) in 246 Chinese children, 3–8 y of age and residing in an area with electronic waste-recycling industries, and their urinary DPD, nor with serum calcium, osteocalcin, or bone alkaline phosphatase levels (Yang et al. 2013). In experimental studies, female rats exposed to cadmium via drinking water (1, 5, and 50 mg/L) from weaning to skeletal maturation, a dose- and time-dependent influence on tibia bone mineral density (BMD) and chemical composition resulted in a weakening in the strength of the tibia (Brzóska et al. 2005).

In the present study, we aimed to elucidate the impact of early-life cadmium exposure on bone-related biomarkers and anthropometry at 9 y of age. This study was conducted in the same Bangladeshi mother–child cohort as our earlier studies, which showed increased cadmium exposure to be associated with reduced anthropometry at birth and at 5 y of age (Gardner et al. 2013; Kippler et al. 2012). Importantly, these earlier studies also indicated more pronounced cadmium-related associations in girls than in boys. Thus, a secondary aim of this study was to elucidate potential gender differences.

Materials and Methods

Study Population

This study was performed in a mother–child cohort in Matlab, Bangladesh, established to assess exposure to arsenic, which frequently occurs at elevated concentrations in well water, and to other environmental pollutants and their impact on pregnancy outcomes and child health and development (Gardner et al. 2013; Kippler et al. 2012; Vahter et al. 2006). The cohort was nested into a population-based food and micronutrient supplementation trial in

Address correspondence to Maria Kippler, Institute of Environmental Medicine, Karolinska Institutet, Box 210, SE-171 77, Stockholm, Sweden. Telephone: 46 8 524 874 07. Email: maria.kippler@ki.se

Supplemental Material is available online (https://doi.org/10.1289/EHP3655).

The authors declare they have no actual or potential competing financial interests.

Received 20 March 2018; Revised 7 February 2019; Accepted 14 February 2019; Published 8 March 2019.

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pregnant women [Maternal and Infant Nutrition Interventions, Matlab (MINIMat)], which recruited 4,436 pregnant women from November 2001 through October 2003 (Persson et al. 2012). The pregnant women were randomly assigned to one of three micronutrient supplementations, starting at gestational week (GW) 14, in combination with food supplementation that they were randomly invited to begin either at enrollment (GW9) or at the time of their choosing (usual care, around GW20), resulting in a total of six intervention groups (Persson et al. 2012). In MINIMat, the eligibility criteria were no severe illness, viable fetus, and gestational age less than 14 weeks (both assessed by ultrasound examination), and consent for participation.

There were 3,625 live births in MINIMat and of these infants, 2,735 were followed up at 4.5 y of age. In order to reduce the number of assessments per child at 4.5 y of age, the children were divided into two groups based on date of birth; group A consisted of 1,432 children born from May 2002 through April 2003 and group B consisted of 1,303 children born from May 2003 through April 2004 (Hawlader et al. 2014). Children in group B were primarily assessed for asthma and allergy, but in a subsample of 640 children, several immune markers were also assessed (Ahmed et al. 2014). The present study included 551 of the 640 children, who were followed up again at 9 y of age (Mannan et al. 2016) and who provided a urine and/or blood sample to be analyzed for cadmium (see Figure S1). Of the 551 children, 504 (91%) and 487 (88%) also had complete outcome and covariate data, respectively, at 9 y of age. A large fraction of these children also had their cadmium exposure assessed at 4.5 y of age (urine $n=496$ and erythrocytes $n=326$) as well as prenatally (maternal urine at GW8 $n=491$ and maternal erythrocytes GW14 $n=238$). For the majority of the exposure time points (see Table S1), the children with complete data did not differ from the children with missing data with regard to their mother's characteristics during pregnancy [age, body mass index (BMI), education, and parity] or their own characteristics at 9 y of age [age, weight, height, and family's socioeconomic status (SES)]. Children with erythrocyte data at 4.5 y of age ($n=326$) were marginally younger (median 8.8 vs. 8.9 y of age) and lighter (21 vs. 22 kg) than children who lacked this data ($n=178$). In addition, children with maternal erythrocyte data ($n=248$) had mothers who were marginally younger (median 26 vs. 27 y of age), were slightly more educated (5.8 vs. 5.1 y of schooling), and had fewer children (1.2 vs. 1.5 children) compared with children who lacked maternal erythrocyte data ($n=256$). Finally, children with erythrocyte cadmium (Ery-Cd) data at all exposure time points ($n=200$) also had mothers who were slightly younger (median 26 vs. 27 y of age) and had fewer children (1.2 vs. 1.5 children) compared with children without Ery-Cd data at all exposure time points ($n=404$).

The study was approved by the ethical review committees at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and by the Regional Ethical Review Board, Stockholm, Sweden. Informed written consent was obtained from the children's guardians. The study was conducted in concordance with the Helsinki Declaration.

Sample Collection

The collection of child urine at 9 and 4.5 y of age and maternal urine in early pregnancy (on average at GW8) has been described in detail elsewhere (Ahmed et al. 2014; Raqib et al. 2017; Vahter et al. 2006). In short, spot urine samples were collected in trace element–free 24-mL polyethylene bottles either at health care facilities in Matlab or at home. Samples were stored in refrigerators until the end of the day when they were transferred to the hospital laboratory for further storage at −70°C. Blood collection was performed at the health care facilities. Children's blood samples at 9 y of age were collected in sodium heparin tubes (Vacuette; Greiner Bio-One International AG), whereas the children's blood samples at 4.5 y of age and the mothers' blood samples at GW14 were collected in lithium heparin tubes (Monovette, Sarstedt AG & Co.). The blood samples were kept cold and they were transported to the hospital laboratory within a couple of hours for immediate separation of plasma and erythrocytes; the different aliquots were thereafter stored at −70°C.

Exposure Assessment

Cadmium was measured in urine and blood (erythrocyte fraction) samples collected from the children at 9 (hereafter referred to as concurrent) and 4.5 y of age and in the mothers at GW8 (urine) and 14 (blood). U-Cd is considered a valid biomarker of chronic cadmium exposure, reflecting the concentration in the kidneys, where about 50% of the cadmium body burden is accumulated with a half-life of decades (Akerstrom et al. 2013). Because about 90–95% of cadmium in blood is stored in the erythrocytes (Carlson and Friberg 1957), Ery-Cd is considered to be a short-term biomarker of cadmium based on the life span of erythrocytes, which is about 2–3 months (Nève 1995).

Cadmium (m/z 111) in urine and erythrocytes was measured with inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce or 7700ce; Agilent Technologies) equipped with an octopole reaction system operating in helium mode (Kippler et al. 2012; Lu et al. 2015) at Karolinska Institutet, Stockholm, Sweden. Prior to the analyses, urine samples were diluted 1:10 in 1% nitric acid (Scharlau, Scharlab, Sentmenat, Spain or Ultrapure Normatom; VWR Chemicals). The erythrocytes samples were diluted 1:25 in an alkali solution [2% (wt:vol) 1-butanol, 0.05% (wt:vol) EDTA, 0.05% (wt:vol) Triton X-100, 1% (wt:vol) ammonium hydroxide (NH_4OH) and 20 μg/L internal standard; Sigma-Aldrich]. Thereafter, the erythrocytes samples were vortex mixed, sonicated for 5 min, and centrifuged at 1,000 rpm for 2 min (MSE centrifuge, Super Minor; MSE (UK) Ltd.). The limit of detection [LOD; three times the standard deviation (SD) of the blank values] when measuring cadmium in child and maternal urine was <0.001 μg/L and <0.004 μg/L, respectively. The corresponding LODs for the erythrocyte measurements were <0.003 μg/kg and <0.006 μg/kg. No samples were below the calculated LODs at any time point. Quality control included the inclusion of several different commercial reference materials for the analysis of each biomarker, and, in general, we found good agreement between the reference concentrations and the obtained concentrations (see Table S2). The majority of the maternal blood samples included in the present study ($n=106$ of 208) had been measured earlier using ICP-MS following acid digestion, and details of this method and related quality control have been described in detail elsewhere (Kippler et al. 2009). The results from the two analytical methods were highly correlated, but the cadmium concentrations from the alkali method were consistently 9% lower than those from the acidic method (Lu et al. 2015). Therefore, to improve the comparability of results across both methods, cadmium concentrations obtained using the acidic method were multiplied by 0.91. In addition to cadmium, we have also measured the concentrations of arsenic in urine both in the children at 9 and 4.5 y of age and in their mothers at GW8 (Ahmed et al. 2014; Raqib et al. 2017; Vahter et al. 2006). The final concentrations of the different elements in urine were adjusted for specific gravity (mean 1.012 for both child and maternal urine) to compensate for variation in urine dilution (Nermell et al. 2008).

Outcomes Measurements at 9 Years of Age

Concentrations of parathyroid hormone (PTH), vitamin D3 (25-hydroxyvitamin D, the inactive form), and osteocalcin, a marker of bone formation, were determined in plasma by chemiluminescence using Cobas e601 (Roche Diagnostics). The mean inter-day coefficient of variation was <5% for PTH and osteocalcin measurements and <8% for vitamin D3. Urinary DPD, a marker of bone resorption, was measured with MicroVue Bone DPD EIA (Quidel) and the inter-assay coefficient of variation was 12%. Calcium in urine (not acidified) was measured using ICP-MS (Agilent 7700ce; Agilent Technologies) and measurements of the reference materials showed good agreement between the reference concentrations and the obtained concentrations (see Table S2). The final urinary DPD and calcium concentrations were adjusted for specific gravity (mean 1.012). Plasma levels of insulin-like growth factor (IGF-1) were measured using the Human IGF-1 ELISA kit (Quantakine ELISA; R&D Systems, Inc.) according to the manufacturer's instructions. The absorbance was measured at 450 nm (reference 650 nm, wavelength correction set at 540) using a microplate reader, and the concentrations were calculated based on the standard curves. The LOD of this ELISA kit was 0.1 ng/mL and all of the plasma samples were above this concentration. The coefficient of variation was 8.3% for IGF-1. The Human IGF-BP3 solid-phase sandwich ELISA (Quantakine ELISA, R&D Systems, Inc.) was used to measure plasma concentration of IGFBP3 according to the manufacturer's instructions. The lowest detection limit was 0.14 ng/mL; the co-efficient of variance of the assay was 6.2%. Plasma TSH was measured at the Clinical Chemistry department at Karolinska University Hospital, Stockholm, through chemiluminescence with Cobas (Roche Diagnostics). Because there was not enough plasma left in all samples, TSH was measured in only a subsample of 299 children for whom the general characteristics (i.e., age, weight, height, family SES) did not differ from the children who did not have enough plasma left.

Child weight and height were measured at 9 y of age by trained nurses. Weight was measured with a daily calibrated digital scale (TANITA HD-318; Tanita Corporation) and height with a regularly calibrated free-standing stadiometer Leicester Height Measure (Seca214). Weight-for-age (WAZ) and height-for-age (HAZ) *Z*-scores were calculated from the measured weight and height using the child growth reference values developed by the WHO (de Onis et al. 2007; WHO 2006). Children with WAZ < -2 SD were graded as underweight and children with HAZ < -2 SD were graded as stunted.

Covariates

Information about the mothers' background characteristics [maternal age, education (number of years of formal schooling), parity, and BMI in early pregnancy, and smoking and alcohol consumption during pregnancy] was obtained at enrollment in MINIMat. None of the mothers smoked or consumed alcohol. Family SES at the follow-up at 9 y of age was estimated via a wealth index, based on information concerning family ownership of assets, housing structure, and dwelling characteristics (Gwatkin et al. 2000). This information was obtained from the Health and Demographic Surveillance System (HDSS) in Matlab, which is administrated by the icddr,b. At the 9-y-of-age follow-up, we also collected information on the number of children in the household and birth order. Season of sampling of blood was categorized as pre-monsoon (January–May), monsoon (June–September), or post-monsoon (October–December). The children's plasma concentration of ferritin, which has been shown in adults to affect cadmium absorption (Berglund et al. 1994), was measured using Cobas e601 autoanalyzer (Roche Diagnostics); the inter-assay coefficient of variation was 1.9%. The cutoff for ferritin deficiency was set at 15 μg/L (WHO 2011b). In addition, hemoglobin (Hb) was measured in whole blood with a HemoCue photometer (HemoCue AB). Anemia was defined as having a Hb concentration <115 g/L (WHO 2011a).

Statistical Analyses

The statistical analyses were performed with Stata 12.1 (StataCorp LLC) and $p < 0.05$ was considered statistically significant. Bivariate associations between cadmium biomarkers (U-Cd and Ery-Cd of the children at 9 and 4.5 y of age and the mothers at GW8 and GW14, respectively), potential covariates (maternal age, level of education and parity at enrollment in MINIMat, BMI in early pregnancy, food and micronutrient supplementation provided during pregnancy, family SES at 9 y of age, number of other children in the household, birth order, and ferritin and Hb at 9 y of age), and outcomes (PTH, osteocalcin, vitamin D3, IGF-1, IGFBP3, and TSH in plasma and DPD and calcium in urine, and WAZ and HAZ at 9 y of age) were, depending on the type of data (continuous or categorical), assessed using either Spearman's rank correlation, Mann-Whitney *U*-test, or Kruskal-Wallis. The associations between different cadmium biomarkers and bone-related biomarkers were also visually inspected using scatter plots with Lowess lines, and we did not observe any indications of nonlinear associations (see Figures S2–S7). Therefore, associations were modeled using multivariable-adjusted linear regression analyses. The different cadmium biomarkers (U-Cd and Ery-Cd) at all time points were left skewed, and because q–q plots and residual versus fitted plots indicated unevenly distributed residuals in some of the multivariable-adjusted models, all the cadmium biomarkers were $\log_2$-transformed. In addition, for the same reason, we also $\log_2$-tranformed urinary arsenic at all time points along with one of the outcomes, urinary calcium.

We applied complete subject analyses at each time point, and we adjusted the models for covariates that were associated with U-Cd and/or Ery-Cd at 9 y of age and with more than three bone-related biomarkers at 9 y of age ($p < 0.05$; see also Table S3); child gender, maternal education (number of years of schooling), family SES (quintiles), and Hb (g/L) at 9 y of age. We also adjusted all the models for urinary arsenic (sum of metabolites; $\log_2$-tranformed) at each exposure time point because arsenic has previously been adversely associated with IGF-1 and IGFBP3 in this cohort (Ahmed et al. 2013; Gliga et al. 2018) and because it may occur in the same dietary sources as cadmium, particularly in rice (Kippler et al. 2016). Thus, in the initial step, hereafter referred to as Model 1, the associations of different cadmium biomarkers with bone-related biomarkers at 9 y of age were adjusted for child gender, maternal education (number of years of schooling), family SES (quintiles) and Hb (g/L) at 9 y of age, and urinary arsenic (sum of metabolites; $\log_2$-tranformed) at each time point of exposure. In a second step (Model 2), we additionally adjusted for maternal BMI in early pregnancy [(kg/m^2) associated with cadmium biomarkers at 9 and 4.5 y of age and with IGF-1 at 9 y of age ($p < 0.05$)] and the food and micronutrient supplementation provided to the mothers during pregnancy [(six groups) associated with cadmium biomarkers at 9 and 4.5 y of age ($p < 0.05$) but not with any bone-related biomarkers].

In sensitivity analyses, in order to distinguish how biomarkers of bone resorption and bone formation influenced each other, the association of children's U-Cd at 9 y of age with urinary DPD (Model 1) was additionally adjusted for osteocalcin, and the association of the U-Cd with osteocalcin was additionally adjusted for DPD. The association of concurrent U-Cd with urinary DPD (Model 1) was further adjusted for urinary or erythrocyte zinc

because zinc may influence potential associations between low-level cadmium exposure and effect markers (Wang et al. 2017). The associations of cadmium biomarkers with vitamin D3 (Model 1) were also additionally adjusted for season of sampling (pre-monsoon, monsoon, post-monsoon). Finally, because Ery-Cd is a biomarker of recent exposure, we combined all three Ery-Cd biomarkers (9 and 4.5 y of age and the mothers during pregnancy) in the same model to elucidate whether this altered their associations with the bone-related biomarkers.

Because our previous studies have indicated sex-specific associations of maternal cadmium exposure with both fetal growth (Kippler et al. 2012) and childhood growth (Gardner et al. 2013), we explored potential interactions between cadmium biomarkers and child gender. This was done by including a multiplicative interaction term (cadmium biomarker × gender) in Model 1 of cadmium biomarkers (9 and 4.5 y of age and during pregnancy) with bone-related biomarkers. Thereafter, we also conducted gender-stratified analyses that were adjusted as Model 1, with the exception of child gender.

Finally, we explored whether the children's concurrent U-Cd was associated with their WAZ and HAZ at 9 y of age. The initial model (Model 1) was adjusted for gender, maternal education (years of formal schooling), family's SES (quintiles), Hb (g/L), and urinary arsenic ($\log_2$-tranformed) at 9 y of age. Thereafter, we conducted several sensitivity analyses where we additionally adjusted for plasma osteocalcin, urinary DPD, plasma vitamin D3, or plasma IGF-1 in order to elucidate whether any of these biomarkers were mediators of any observed association. We also tested for a potential interaction with gender by inclusion of a multiplicative interaction term [concurrent U-Cd ($\log_2$) × gender] in all models. The models were also stratified by gender.

Results

General Characteristics, Cadmium Exposure, and Bone-Related Biomarkers

The general characteristics of the children, 248 (49%) boys and 256 (51%) girls, included in the present study are shown in Table 1. Nearly one-fourth of the children (23%) were stunted and 41% were underweight at 9 y of age. About 22% of the children had a low vitamin D3 concentration in plasma (<50 nmol/L), and 16% had a Hb concentration <115 g/L (anemic), whereas only one child had a serum ferritin value <15 μg/L (indicating depleted iron stores). Girls were, in general, slightly lighter and shorter compared with boys (Table 1). In addition, girls had slightly higher levels of PTH, osteocalcin, IGF-1, and IGFBP3 compared with boys, whereas boys had higher levels of vitamin D3 and urinary calcium compared with girls.

The concentrations of U-Cd, a marker of long-term exposure, increased with age (see Table S4). Median U-Cd was 0.28 μg/L at 9 y of age and 0.22 μg/L at 4.5 y of age, and they were moderately correlated ($r_S = 0.52$, $p < 0.001$), whereas the U-Cd in the mothers in early pregnancy was almost twice as high (0.55 μg/L). The Ery-Cd concentrations (reflecting ongoing exposure), on the other hand, were quite similar irrespective of age (median: 0.90 μg/kg at 9 y of age, 0.94 μg/kg at 4.5 y of age, and 0.89 μg/kg in the mothers at GW14). Ery-Cd at 9 and 4.5 y of age were strongly correlated ($r_S = 0.68$, $p < 0.001$). Girls had slightly higher U-Cd compared with boys, but not Ery-Cd, at 9 and 4.5 y of age (see Table S4). The children's U-Cd concentrations were weakly correlated with their urinary arsenic (sum of arsenic metabolites) concentrations ($r_S = 0.12$, $p < 0.01$ at 9 y of age and $r_S = 0.12$, $p = 0.011$ at 4.5 y of age). In bivariate analyses

Table 1. Main characteristics of 504 children at 9 y of age (248 boys and 256 girls) and of their mothers during pregnancy.

Characteristics	All children	Boys	Girls	*p*-Value[a]
Children at 9 y of age				
Age (y)	8.9 ± 0.13	8.9 ± 0.12	8.9 ± 0.15	0.99
Weight (kg)	22 ± 3.5	22 ± 3.4	22 ± 3.6	0.019
WAZ (*z*-score)	−1.7 ± 1.0	−1.7 ± 1.1	−1.8 ± 0.99	0.77
Height (cm)	124 ± 5.3	124 ± 5.4	123 ± 5.1	0.030
HAZ (*z*-score)	−1.4 ± 0.89	−1.3 ± 0.91	−1.4 ± 0.86	0.12
SES (% in each quintile)	21/20/19/19/20	22/20/19/16/24	21/20/20/23/16	0.75
Children in household (*n*)	2.8 ± 0.99	2.9 ± 1.1	2.8 ± 0.87	0.65
Birth order	2.3 ± 1.3	2.4 ± 1.4	2.2 ± 1.1	0.72
Hemoglobin (g/dL)	13 (11–14)	13 (11–14)	12 (11–14)	0.69
Ferritin (ng/mL)	55 (24–110)	55 (23–105)	56 (25–121)	0.30
Erythrocyte zinc (mg/kg)	9.3 (6.8–11.6)	9.4 (6.9–11.7)	9.2 (6.8–11.3)	0.30
Urinary zinc (mg/L)[b]	0.20 (0.073–0.46)	0.20 (0.086–0.45)	0.21 (0.061–0.50)	0.21
Urinary calcium (mg/L)[b]	15 (2.7–65)	17 (3.0–71)	13 (2.1–61)	0.019
Urinary DPD (nmol/L)[b]	154 ± 99	155 ± 98	153 ± 100	0.89
PTH (pg/mL)	38 ± 13	36 ± 12	41 ± 14	<0.001
Osteocalcin (ng/mL)	100 ± 28	97 ± 27	104 ± 28	0.0037
Vitamin D3 (nmol/L)	64 ± 17	66 ± 17	61 ± 17	<0.001
IGF-1 (ng/mL)	98 ± 34	88 ± 29	107 ± 35	<0.001
IGFBP3 (ng/mL)	2852 ± 835	2730 ± 796	2970 ± 857	<0.001
TSH (mE/L)[c]	3.1 ± 1.6	3.0 ± 1.6	3.2 ± 1.7	0.15
Season blood sampling (%)[d]	32/35/33	31/36/33	34/34/32	0.77
Mothers during pregnancy				
Age (y)	26 ± 5.9	26 ± 6.0	26 ± 5.7	0.80
BMI (kg/m2)	21 ± 2.9	21 ± 2.8	20 ± 2.9	0.31
Education (y)	5.4 ± 3.9	5.6 ± 3.9	5.3 ± 3.8	0.24
Parity (number of children)	1.4 ± 1.3	1.4 ± 1.4	1.3 ± 1.1	0.71
Supplementation group (%)[f]	19/18/16/15/18/14	19/19/16/15/16/15	18/17/16/15/19/14	0.97

Note: Values represent Mean ± standard deviation or median (5th and 95th percentile). BMI, body mass index; DPD, deoxypyridinoline; HAZ, height-for-age z-core; IGF-1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor binding protein 3; PTH, parathyroid hormone; SES, socioeconomic status; TSH, thyroid stimulating hormone; WAZ, weight-for-age z-score.
[a]Derived by Mann-Whitney *U*-Test or Chi Square Test.
[b]Adjusted for urinary specific gravity, mean of 1.012.
[c]Only available for a subsample of 299; 146 boys and 153 girls.
[d]Defined as pre-monsoon (January–May), monsoon (June–September), or post-monsoon (October–December).
[e]Six different supplementation groups [combination of food (two groups) and micronutrient supplementation (three groups)].

(see Table S3), U-Cd at 9 y of age was significantly positively correlated with PTH and DPD and inversely with vitamin D3, urinary calcium, WAZ, and HAZ. Ery-Cd at 9 y of age was significantly positively correlated with osteocalcin and inversely correlated with DPD, IGF-1, Hb, and ferritin.

Cadmium Exposures and Bone-Related Biomarkers at 9 Years of Age

In the multivariable-adjusted cross-sectional analyses (Table 2, Model 1), a doubling of the children's concurrent U-Cd was associated with a mean increase in their plasma osteocalcin of 2.7 ng/mL [95% confidence interval (CI): −0.26, 5.6] and in their urinary DPD of 22 nmol/L (95% CI: 12, 32). A doubling of the U-Cd was also associated with a mean decrease in urinary calcium of 16% (95% CI: −31%, −1.2%). Children's concurrent U-Cd and their U-Cd at 4.5 y of age were both associated with a mean decrease in vitamin D3 [−2.6 nmol/L (95% CI: −4.3, −0.83) and −2.2 nmol/L (95% CI: −3.9, −0.60), respectively]. Similar inverse trends were observed with IGF-1 [−2.6 ng/mL (95% CI: −5.9, 0.74) and −2.3 ng/mL (95% CI: −5.3, 0.83), respectively] and IGFBP3 [−49 ng/mL (95% CI: −138, 39) and −64 ng/mL (95% CI: −146, 19), respectively]. Further adjustment for maternal BMI in early pregnancy and food and micronutrient supplementation during pregnancy (Model 2) had no impact on the estimates for any cadmium biomarker and urinary DPD or urinary calcium, whereas it marginally decreased the estimates for some cadmium biomarker and osteocalcin (15%), vitamin D3 (≤12%), and IGF-1 (≤8%). The additional adjustment increased the estimate for the association of concurrent U-Cd and IGFBP-3 by 14%, whereas it marginally decreased the association with U-Cd at 4.5 y of age (5%). None of the children's U-Cd biomarkers was associated with PTH or TSH (Table 2). Maternal U-Cd in early pregnancy was not associated with any of the bone-related biomarkers.

A doubling in the children's concurrent Ery-Cd was associated with a mean increase in osteocalcin of 5.7 ng/mL (95% CI: 1.8, 9.5), whereas no association was observed with DPD (Model 1). Likewise, every doubling of the children's concurrent Ery-Cd was associated with a mean decrease in urinary calcium of 16% (95% CI: −37%, 3.7%). A doubling of Ery-Cd, both concurrent and at 4.5 y of age, was associated with a mean decrease in vitamin D3 [−2.4 nmol/L (95% CI: −4.8, −0.098) and −4.4 nmol/L (95% CI: −6.8, −1.9), respectively]. Maternal Ery-Cd during pregnancy was associated only with TSH; a doubling in Ery-Cd decreased TSH of −0.26 mE/L (95% CI: −0.46, −0.052), and a similar

Table 2. Multivariable-adjusted linear regression models of cadmium biomarkers (concentrations in urine and erythrocytes at 9 or 4.5 y of age or of the mothers during pregnancy; $\log_2$-transformed) with bone-related biomarkers at 9 y of age.

		Exposure at 9 y of age		Exposure at 4.5 y of age		Exposure prenatally	
Cadmium biomarkers	Models	B (95% CI)	*p*-Value	B (95% CI)	*p*-Value	B (95% CI)	*p*-Value
Urinary cadmium ($\log_2$)[a]							
PTH (pg/mL)	Model 1	0.50 (−0.88, 1.9)	0.48	−0.50 (−1.8, 0.78)	0.44	0.32 (−0.79, 1.4)	0.57
	Model 2	0.36 (−1.0, 1.8)	0.61	−0.55 (−1.8, 0.75)	0.41	0.32 (−0.82, 1.4)	0.58
Osteocalcin (ng/mL)	Model 1	2.7 (−0.26, 5.6)	0.073	0.62 (−2.1, 3.4)	0.66	1.0 (−1.4, 3.4)	0.40
	Model 2	2.3 (−0.66, 5.3)	0.13	0.38 (−2.4, 3.1)	0.79	0.91 (−1.5, 3.3)	0.45
DPD (nmol/L)	Model 1	22 (12, 32)	<0.001	4.2 (−5.6, 14)	0.40	0.23 (−8.2, 8.6)	0.96
	Model 2	23 (12, 33)	<0.001	4.0 (−5.9, 14)	0.43	0.60 (−7.8, 9.0)	0.89
Urinary calcium (mg/L; $\log_2$)	Model 1	−0.16 (−0.31, −0.012)	0.034	−0.062 (−0.20, 0.080)	0.39	−0.0036 (−0.13, 0.12)	0.95
	Model 2	−0.16 (−0.31, −0.0019)	0.047	−0.061 (−0.20, 0.082)	0.41	−0.0032 (−0.13, 0.12)	0.96
Vitamin D3 (nmol/L)	Model 1	−2.6 (−4.3, −0.83)	0.004	−2.2 (−3.9, −0.60)	0.008	0.91 (−0.52, 2.3)	0.21
	Model 2	−2.3 (−4.1, −0.53)	0.011	−2.1 (−3.7, −0.46)	0.012	0.96 (−0.47, 2.4)	0.19
IGF-1 (ng/mL)	Model 1	−2.6 (−5.9, 0.74)	0.13	−2.3 (−5.3, 0.83)	0.15	0.22 (−2.4, 2.9)	0.87
	Model 2	−2.4 (−5.8, 0.87)	0.15	−2.2 (−5.3, 0.84)	0.15	0.12 (−2.5, 2.8)	0.93
IGFBP3 (ng/mL)	Model 1	−49 (−138, 39)	0.27	−64 (−146, 19)	0.13	11 (−60, 82)	0.76
	Model 2	−56 (−145, 34)	0.22	−61 (−144, 22)	0.15	7.6 (−64, 79)	0.83
TSH (mE/L)[b]	Model 1	−0.091 (−0.32, 0.14)	0.43	−0.054 (−0.27, 0.16)	0.62	−0.11 (−0.29, 0.070)	0.23
	Model 2	−0.10 (−0.34, 0.13)	0.38	−0.049 (−0.27, 0.17)	0.66	−0.098 (−0.28, 0.084)	0.29
Erythrocyte cadmium ($\log_2$)[c]							
PTH (pg/mL)	Model 1	−0.26 (−2.1, 1.6)	0.78	−0.40 (−2.2, 1.4)	0.67	−0.12 (−1.5, 1.2)	0.86
	Model 2	−0.38 (−2.2, 1.5)	0.69	−0.73 (−2.6, 1.2)	0.45	0.0045 (−1.4, 1.4)	0.99
Osteocalcin (ng/mL)	Model 1	5.7 (1.8, 9.5)	0.004	1.4 (−2.9, 5.7)	0.53	−0.99 (−4.5, 2.5)	0.56
	Model 2	5.3 (1.4, 9.2)	0.008	0.68 (−3.7, 5.1)	0.76	−1.6 (−5.1, 1.9)	0.38
DPD (nmol/L)	Model 1	1.3 (−12, 15)	0.85	8.0 (−7.3, 23)	0.30	7.9 (−5.2, 21)	0.24
	Model 2	0.37 (−13, 14)	0.96	8.2 (−7.6, 24)	0.31	6.7 (−6.5, 20)	0.32
Urinary calcium (mg/L; $\log_2$)	Model 1	−0.16 (−0.37, 0.037)	0.11	−0.097 (−0.31, 0.12)	0.38	−0.11 (−0.28, 0.069)	0.24
	Model 2	−0.15 (−0.36, 0.051)	0.14	−0.095 (−0.32, 0.13)	0.40	−0.090 (−0.27, 0.086)	0.32
Vitamin D3 (nmol/L)	Model 1	−2.4 (−4.8, −0.098)	0.041	−4.4 (−6.8, −1.9)	0.001	0.74 (−1.3, 2.8)	0.47
	Model 2	−2.1 (−4.4, 0.25)	0.079	−4.0 (−6.5, −1.5)	0.002	0.83 (−1.2, 2.9)	0.43
IGF-1 (ng/mL)	Model 1	0.87 (−3.5, 5.2)	0.70	−2.0 (−6.7, 2.6)	0.39	0.42 (−3.3, 4.1)	0.83
	Model 2	0.93 (−3.4, 5.3)	0.68	−1.5 (−6.3, 3.2)	0.53	0.12 (−3.6, 3.8)	0.95
IGFBP3 (ng/mL)	Model 1	21 (−98, 140)	0.73	−68 (−201, 65)	0.32	39 (−64, 142)	0.46
	Model 2	17 (−103, 136)	0.78	−74 (−210, 62)	0.29	26 (−79, 131)	0.63
TSH (mE/L)[d]	Model 1	−0.25 (−0.57, 0.071)	0.13	−0.14 (−0.48, 0.20)	0.42	−0.23 (−0.43, −0.033)	0.023
	Model 2	−0.28 (−0.61, 0.048)	0.094	−0.19 (−0.54, 0.17)	0.30	−0.26 (−0.46, −0.052)	0.014

Note: Model 1 was adjusted for child gender, maternal education (years of schooling), family's socioeconomic status and child hemoglobin at 9 y of age, and urinary arsenic (sum of metabolites, $\log_2$-transformed) at each time point of exposure. Model 2 was additionally adjusted for maternal BMI (kg/m^2) during early pregnancy and the food and micronutrient supplementation provided during pregnancy (six groups). CI, confidence interval; DPD, deoxypyridinoline; IGF-1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor binding protein 3; PTH, parathyroid hormone; TSH, thyroid stimulating hormone.

[a]The models with urinary cadmium contained 504 children at 9 y of age, 496 children at 4.5 y of age, and 491 prenatally.

[b]TSH was measured only in a subsample of 299 in relation to urinary cadmium at 9 y of age, 296 at 4.5 y of age, and 290 prenatally.

[c]The models with erythrocyte cadmium contained 487 children at 9 y of age, 326 children at 4.5 y of age, and 248 prenatally.

[d]TSH was measured only in a subsample of 297 in relation erythrocyte cadmium at 9 y of age, 223 at 4.5 y of age, and 175 prenatally.

Table 3. Multivariable-adjusted linear regression models of cadmium biomarkers (concentrations in urine and erythrocytes at 9 or 4.5 y of age or of the mothers during pregnancy; $\log_2$-transformed) with bone-related biomarkers at 9 y of age stratified by gender.

	Exposure at 9 y of age			Exposure at 4.5 y of age			Exposure prenatally		
Biomarkers 9 y of age	Boys [B (95% CI)]	Girls [B (95% CI)]	p_{int}[a]	Boys [B (95% CI)]	Girls [B (95% CI)]	p_{int}[a]	Boys [B (95% CI)] B (95% CI)	Girls [B (95% CI)]	p_{int}[a]
U-Cd ($\log_2$)[b,c]									
PTH (pg/mL)	1.3 (−0.52, 3.1)	−0.32 (−2.4, 1.8)	0.22	0.068 (−1.6, 1.7)	−1.2 (−3.2, 0.78)	0.17	−0.91 (−2.4, 0.53)	1.6 (−0.093, 3.4)	0.024
Osteocalcin (ng/mL)	−4.3 (−8.5, −0.080)	9.4 (5.4, 13)	0.001	−3.3 (−7.1, 0.54)	4.3 (0.41, 8.3)	0.016	−0.051 (−3.4, 3.3)	1.7 (−1.7, 5.2)	0.57
DPD (nmol/L)	22 (7.5, 37)	21 (7.3, 36)	0.97	12 (−1.7, 25)	−3.7 (−18, 10)	0.11	0.74 (−11, 12)	1.1 (−11, 13)	0.82
Urinary Ca (mg/L)[d]	−0.19 (−0.41, 0.031)	−0.14 (−0.35, 0.075)	0.61	−0.032 (−0.23, 0.17)	−0.091 (−0.30, 0.11)	0.86	0.044 (−0.13, 0.22)	−0.054 (−0.23, 0.12)	0.46
Vitamin D3 (nmol/L)	−2.5 (−5.2, 0.14)	−2.7 (−5.1, −0.36)	0.86	−3.5 (−5.8, −1.1)	−0.88 (−3.2, 1.4)	0.086	0.64 (−1.5, 2.8)	1.2 (−0.79, 3.2)	0.76
IGF-1 (ng/mL)	−5.3 (−9.5, −1.0)	−0.84 (−5.9, 4.2)	0.17	−2.1 (−5.9, 1.7)	−2.8 (−7.7, 2.0)	0.69	0.75 (−2.6, 4.1)	0.036 (−4.1, 4.2)	0.74
IGFBP3 (ng/mL)	−62 (−188, 64)	−34 (−160, 93)	0.75	−64 (−177, 49)	−78 (−199, 44)	0.79	99 (0.83, 197)	−70 (−173, 34)	0.045
TSH (mE/L)[e]	−0.012 (−0.35, 0.33)	−0.16 (−0.48, 0.16)	0.43	−0.16 (−0.49, 0.17)	0.047 (−0.25, 0.35)	0.50	−0.037 (−0.31, 0.23)	−0.17 (−0.42, 0.079)	0.41
Ery-Cd ($\log_2$)[b,f]									
PTH (pg/mL)	0.46 (−1.8, 2.7)	−1.1 (−4.1, 1.8)	0.23	1.8 (−0.63, 4.3)	−2.6 (−5.3, 0.15)	0.029	0.97 (−0.70, 2.6)	−1.2 (−3.3, 0.97)	0.16
Osteocalcin (ng/mL)	3.6 (−1.8, 9.0)	7.9 (2.3, 13)	0.75	−2.6 (−8.5, 3.3)	4.7 (−1.4, 11)	0.37	−1.0 (−5.8, 3.8)	−0.80 (−6.0, 4.4)	0.89
DPD (nmol/L)	6.0 (−13, 25)	−4.6 (−25: 15)	0.37	25 (3.3, 47)	−10 (−31, 11)	0.013	1.7 (−16, 20)	13 (−5.6, 32)	0.24
Urinary Ca (mg/L)[d]	−0.26 (−0.54, 0.013)	−0.053 (−0.35, 0.25)	0.13	−0.20 (−0.51, 0.12)	−0.0055 (−0.31, 0.30)	0.38	−0.18 (−0.43, 0.071)	−0.033 (−0.28, 0.21)	0.52
Vitamin D3 (nmol/L)	−3.5 (−6.8, −0.10)	−1.5 (−4.8, 1.8)	0.36	−5.4 (−9.1, −1.8)	−3.4 (−6.7, −0.014)	0.53	1.7 (−1.4, 4.8)	−0.20 (−3.0, 2.6)	0.34
IGF-1 (ng/mL)	0.17 (−5.2, 5.6)	1.5 (−5.5, 8.5)	0.77	−4.6 (−10, 1.3)	−0.37 (−7.6, 6.9)	0.29	2.5 (−2.3, 7.3)	−1.6 (−7.3, 4.1)	0.43
IGFBP3 (ng/mL)	124 (−36, 284)	−80 (−258, 98)	0.091	−36 (−219, 148)	−104 (−298, 89)	0.67	162 (25, 300)	−95 (−248, 59)	0.015
TSH (mE/L)[g]	−0.52 (−1.0, −0.0093)	−0.062 (−0.49, 0.37)	0.18	−0.54 (−1.1, −0.020)	0.24 (−0.21, 0.69)	0.038	−0.21 (−0.53, 0.10)	−0.22 (−0.48, 0.042)	0.94

Note: CI, confidence interval; DPD, deoxypyridinoline; Ery-Cd, erythrocyte cadmium; IGF-1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor binding protein 3; p_{int}, p for interaction; PTH, parathyroid hormone; TSH, thyroid stimulating hormone; U-Cd, urinary cadmium.

[a]Models included a multiplicative interaction term (cadmium biomarker × gender) and were adjusted for maternal education (years of schooling), family's socioeconomic status, child hemoglobin at 9 y of age, and urinary arsenic (sum of metabolites, $\log_2$-transformed) at each time point of exposure.

[b]Models adjusted for maternal education (years of schooling), family's socioeconomic status, child hemoglobin at 9 y of age, and urinary arsenic (sum of metabolites, $\log_2$-transformed) at each time point of exposure.

[c]The models with urinary cadmium contained 248 boys and 256 girls at 9 y of age, 245 boys and 251 girls at 4.5 y of age, and 240 boys and 251 girls prenatally.

[d]$\log_2$-transformed.

[e]TSH was available for only a subsample of 146 boys and 153 girls in relation to urinary cadmium at 9 y of age, 144 boys and 152 girls at 4.5 y of age, and 141 boys and 149 girls prenatally.

[f]The models with erythrocyte cadmium contained 242 boys and 245 girls at 9 y of age, 160 boys and 166 girls at 4.5 y of age, and 127 boys and 121 girls prenatally.

[g]TSH was available for only a subsample of 146 boys and 151 girls in relation to erythrocyte cadmium at 9 y of age, 111 boys and 112 girls at 4.5 y of age, and 89 boys and 86 girls prenatally.

Table 4. Multivariable-adjusted linear regression analyses of children's concurrent urinary cadmium (log_2-transformed) with weight-for-age and height-for-age z-scores at 9 y of age in all children ($n = 504$) as well as in boys ($n = 248$) and girls ($n = 256$) separately.

	All children		Boys		Girls		
Outcomes	B (95% CI)	p-Value	B (95% CI)	p-Value	B (95% CI)	p-Value	p_{int}[a]
WAZ							
Model 1[b]	−0.087 (−0.19, 0.019)	0.11	−0.13 (−0.29, 0.036)	0.13	−0.046 (−0.19, 0.096)	0.53	0.31
Model 2[c]	−0.097 (−0.20, 0.0083)	0.071	−0.099 (−0.26, 0.062)	0.23	−0.061 (−0.21, 0.088)	0.42	0.56
Model 3[d]	−0.096 (−0.20, 0.011)	0.080	−0.14 (−0.31, 0.020)	0.086	−0.043 (−0.19, 0.10)	0.56	0.31
Model 4[e]	−0.11 (−0.21, −0.0039)	0.042	−0.14 (−0.30, 0.019)	0.083	−0.074 (−0.22, 0.068)	0.30	0.31
Model 5[f]	−0.055 (−0.15, 0.043)	0.27	−0.044 (−0.19, 0.11)	0.57	−0.037 (−0.17, 0.096)	0.58	0.58
HAZ							
Model 1[b]	−0.044 (−0.14, 0.047)	0.34	−0.073 (−0.21, 0.065)	0.30	−0.0095 (−0.13, 0.11)	0.88	0.38
Model 2[c]	−0.056 (−0.15, 0.034)	0.22	−0.50 (−0.19, 0.088)	0.48	−0.045 (−0.17, 0.083)	0.49	0.77
Model 3[d]	−0.059 (−0.15, 0.034)	0.21	−0.090 (−0.23, 0.050)	0.21	−0.021 (−0.15, 0.10)	0.74	0.38
Model 4[e]	−0.048 (−0.14, 0.044)	0.30	−0.068 (−0.21, 0.071)	0.33	−0.023 (−0.15, 0.10)	0.72	0.39
Model 5[f]	−0.018 (−0.10, 0.067)	0.68	−0.0044 (−0.13, 0.12)	0.95	−0.0021 (−0.12, 0.11)	0.97	0.67

Note: CI, confidence interval; HAZ, height-for-age z-score; p_{int}, p for interaction; WAZ, weight-for-age z-score.
[a]Multiplicative interaction term [urinary cadmium (log_2) × gender)] included in each respective model defined below.
[b]Model 1 adjusted for sex, maternal education (number of years of schooling), family's socioeconomic status (quintiles), children's hemoglobin (g/L) at 9 y of age, and children's urinary arsenic at 9 y of age (sum of metabolites, log_2-transformed).
[c]Model 2 additionally adjusted for osteocalcin (ng/mL) at 9 y of age.
[d]Model 3 additionally adjusted for urinary DPD (nmol/L) at 9 y of age.
[e]Model 4 additionally adjusted for vitamin D3 (nmol/L) at 9 y of age.
[f]Model 5 additionally adjusted for IGF-1 (ng/mL) at 9 y of age.

tendency was observed for childhood Ery-Cd [−0.28 mE/L (95% CI: −0.61, 0.048) and −0.19 mE/L (95% CI: −0.54, 0.17) at 9 and 4.5 y of age, respectively]. Additional adjustment of all these associations above (Model 2) had marginal impact on the different estimates of Ery-Cd (≤13%). None of the Ery-Cd biomarkers was associated with PTH, DPD, IGF-1, or IGFBP3 (Table 2).

In sensitivity analyses, we additionally adjusted the model of children's concurrent U-Cd and urinary DPD (Model 1) for osteocalcin and *vice versa*. The association between U-Cd and urinary DPD remained essentially unchanged (B = 19 nmol/L; 95% CI: 9.5, 29; $p < 0.001$), whereas the association between U-Cd and osteocalcin decreased markedly (B = 1.0 ng/mL; 95% CI: −1.9, 3.9; $p = 0.48$). As a second sensitivity analyses, we additionally adjusted the association of concurrent U-Cd with urinary DPD (Model 1) for urinary zinc (B = 22 nmol/L; 95% CI: 12, 32; $p < 0.001$) or erythrocyte zinc (B = 23 nmol/L; 95% CI: 12, 33; $p < 0.001$), which did not alter the estimate. Furthermore, additional adjustment of Model 1 (Table 2) for season of blood sampling strengthened the inverse associations of both concurrent and 4.5-y-of-age U-Cd with vitamin D3 by about 14% (B = −2.6 nmol/L; 95% CI: −4.3, −0.82; $p = 0.004$ and B = −2.4 nmol/L; 95% CI: −4.0, −0.76; $p = 0.004$), respectively. Similarly, further adjustment for season of sampling strengthened the inverse associations of concurrent and 4.5-y-of-age Ery-Cd with vitamin D3 to about the same extent (B = −2.5 nmol/L; −4.8, −0.11; $p = 0.040$ and B = −4.5 nmol/L; −7.0, −1.9; $p = 0.001$), respectively.

Because Ery-Cd reflects ongoing exposure, we performed a sensitivity analysis were Ery-Cd at all the three time points (9 and 4.5 y of age and during pregnancy) were combined in the same model ($n = 200$; see also Table S5). As in the single exposure model, concurrent Ery-Cd was positively associated with osteocalcin (B = 7.7 mg/mL; 95% CI: −2.5, 18) and inversely associated with urinary calcium (B = −35%; 95% CI: −85%, 14%). Both concurrent and 4.5-y-of-age Ery-Cd remained inversely associated with vitamin D3 (B = −4.1 nmol/L; 95% CI: −9.7, 1.4 and B = −3.6 nmol/L; 95% CI: −8.2, 0.90, respectively). In addition, the inverse association of maternal Ery-Cd during pregnancy with TSH remained (B = −0.27 mE/L; 95% CI: −.50, −0.042), whereas there was no longer any tendency of association for childhood Ery-Cd. The only new association that emerged was that between maternal Ery-Cd during pregnancy and child urinary DPD (B = 15 nmol/L; 95% CI: −0.047, 30), and there was a similar estimate for 4.5-y-of-age Ery-Cd (B = 16 nmol/L; 95% CI: −14, 46).

Gender Differences

There appeared to be a gender difference in the multivariable-adjusted association of concurrent U-Cd and osteocalcin ($p_{interaction} = 0.001$; Table 3); in boys, a doubling of U-Cd was associated with a mean decrease in osteocalcin of −4.3 ng/mL (95% CI: −8.5, −0.080), whereas, in girls, it was associated with an increase of 9.4 ng/mL (95% CI: 5.4, 13). The same pattern was observed for U-Cd at 4.5 y of age ($p_{interaction} = 0.016$; Table 3), where a doubling was associated with a mean decrease in osteocalcin of −3.3 ng/mL (95% CI: −7.1, 0.54) in boys and an increase of 4.3 ng/mL (95% CI: 0.41, 8.3) in girls. For urinary DPD, a doubling of Ery-Cd at 4.5 y of age was associated with a mean increase in urinary DPD of 25 nmol/L (95% CI: 3.3, 47) in boys and a mean decrease in urinary DPD of −10 nmol/L (95% CI: −31, 11) in girls ($p_{interaction} = 0.013$). However, no other cadmium biomarker (childhood or prenatal) showed any gender difference for DPD.

For PTH, a doubling of maternal U-Cd was associated with a slight mean decrease in PTH of −0.91 pg/mL (95% CI: −2.4, 0.53) in boys and a mean increase of 1.6 pg/mL (95% CI: −0.093, 3.4) in girls ($p_{interaction} = 0.024$), whereas maternal Ery-Cd showed the opposite pattern with an increase of 0.97 pg/mL (95% CI: −0.70, 2.6) in boys and a decrease of −1.2 pg/mL (95% CI: −3.3, 0.97) in girls ($p_{interaction} = 0.16$). In addition, all childhood cadmium biomarkers showed positive associations in boys and inverse associations in girls, although with significant interaction term of 0.029 for only Ery-Cd at 4.5 y of age [1.8 pg/mL (95% CI: −0.63, 4.3) and −2.6 pg/mL (95% CI: −5.3, 0.15) for boys and girls, respectively].

The associations of children's Ery-Cd with TSH appeared to differ by gender ($p_{interaction} = 0.038$ and 0.18 for exposure at 4.5 and 9 y of age, respectively; Table 3). A doubling of Ery-Cd at 4.5 y of age was associated with a mean decrease in TSH of −0.54 mE/L (95% CI: −1.1, −0.020) in boys and a mean increase in TSH of 0.24 mE/L (95% CI: −0.21, 0.69) in girls. There was a similar estimate for concurrent Ery-Cd and TSH in boys (−0.52 mE/L, 95% CI: −1.0, −0.009), whereas there was no association in girls (−0.062 mE/L, 95% CI: −0.49, 0.37).

There appeared to be gender differences in the associations of both maternal U-Cd and Ery-Cd during pregnancy with child IGFBP3 ($p_{interaction} = 0.045$ and 0.015, respectively; Table 3). A doubling of maternal U-Cd was associated with a mean increase in IGFBP3 of 99 ng/mL (95% CI: 0.83, 197) in boys and a mean decrease of −70 ng/mL (95% CI: −173, 34) in girls. A similar pattern was found with maternal Ery-Cd [162 ng/mL (95% CI: 25, 300) and −95 ng/mL (95% CI: −248, 59) for boys and girls, respectively]. The associations of U-Cd or Ery-Cd biomarkers with urinary calcium, vitamin D3, or IGF-1 did not differ by gender (Table 3).

Cadmium Exposure and Anthropometry at 9 Years of Age

Concerning the evaluation of cadmium and anthropometric measures at 9 y of age, the multivariable-adjusted cross-sectional analyses (Table 4; Model 1, including all children) revealed that a doubling of concurrent U-Cd was associated with a mean decrease in WAZ of −0.087 *z*-score (95% CI: −0.19; 0.019) and in HAZ of −0.044 *z*-score (95% CI: −0.14; 0.047), but the confidence intervals were wide. The association appeared to strengthen after further adjustment for vitamin D3 (Table 4), after which a doubling of concurrent U-Cd was associated with a mean decrease in WAZ of −0.11 *z*-score (95% CI: −0.21; −0.004). Importantly, further adjustment for IGF-1 annulled the association of concurrent U-Cd and WAZ (−0.055 *z*-score; 95% CI: −0.15; 0.043). We found no indications of any interaction between the children's concurrent U-Cd and gender in relation to either WAZ or HAZ, although the estimate for U-Cd on WAZ on was about twice as high in boys as in girls (Table 4).

Discussion

The period of childhood and adolescence is important for linear growth and acquisition of adequate skeletal bone mineral density. We found, for the first time, that cadmium exposure during childhood was associated with multiple alterations in bone-related biomarkers in prepubertal children. Specifically, U-Cd, a marker of chronic exposure, was positively associated with urinary DPD and plasma osteocalcin and inversely associated with plasma vitamin D3. Stratification by child gender indicated that the positive association of cadmium exposure with osteocalcin occurred only in girls, whereas the association was inverse in boys ($p_{interaction} = 0.001$). Moreover, increasing U-Cd concentrations were associated with decreasing WAZ at 9 y of age in all children, although the confidence interval was wide, and, interestingly, further adjustment for IGF-1 attenuated this association. Indeed, children's U-Cd concentrations were inversely associated with IGF-1, also with wide confidence intervals.

Bone resorption and formation, two continuous lifelong processes of bone metabolism, are tightly coupled through several feedback mechanisms (Raggatt and Partridge 2010). In children, this process is further complicated by bone growth, consisting of both linear growth and bone accrual. Markers of bone remodeling are highly expressed during the first 3 y of life, followed by a lower expression until the start of puberty, when biomarkers of bone remodeling are again highly upregulated (Jürimäe 2010). Much lower expression levels are seen during adulthood. Our results showed that a doubling of U-Cd was associated with a mean increase in urinary DPD, a marker of bone resorption, of 22 nmol/L (95% CI: 12, 32), corresponding to 22% of the SD. This is in concordance with the finding of a cross-sectional study of children in Lahore, Pakistan, 8–12 y of age, with twice as high median U-Cd concentration (∼0.56 μg/L) (Sughis et al. 2011) compared with the rural Bangladeshi children in the present study. However, they did not measure any biomarker of bone formation in the Pakistani children.

Experimental studies in rats have shown that even low-level exposure to cadmium during phases of intensive bone growth and development disturbs bone mineralization, resulting in weakened mechanical properties (Brzóska et al. 2005). Both *in vitro* and *in vivo* studies have shown that cadmium stimulates osteoclast formation, leading to bone resorption (Rodríguez and Mandalunis 2016; Wilson et al. 1996). In the present study, the association with DPD seemed to start very early in life because a doubling in maternal Ery-Cd during pregnancy was associated with a mean increase in child urinary DPD of 15 nmol/L (95% CI: −0.047; 30) in the combined exposure analysis, adjusted for childhood exposure. Thus, it may be hypothesized that elevated cadmium exposure leads to an increase in child bone resorption, prompting an increase in bone formation through feedback mechanisms. Indeed, we did find that increased cadmium exposure during childhood was associated with increased levels of osteocalcin, a marker of bone formation, although this association differed by gender ($p_{interaction} = 0.001$). In girls, a doubling of U-Cd was associated with a mean increase in osteocalcin of 9.4 ng/mL (95% CI: 5.4, 13; corresponding to 0.34 SD), whereas in boys, it was associated with a mean decrease in osteocalcin of 3.7 ng/ml (95% CI: −8.5, −0.080; corresponding to about 0.14 SD).

The underlying mechanism for this gender difference is unknown. The studied children were between 8.7 and 10 y of age, and some of the girls might have reached puberty. Unfortunately, we had no data on puberty development or measures of serum estradiol, which has previously been positively associated with osteocalcin in prepubertal Hungarian girls (Csakvary et al. 2013). However, in another subsample of girls from the MINIMat birth cohort ($n = 513$), Tanner breast development staging, assessed at 10 y of age, showed that 11% of the girls had reached stage 2 or more (Svefors et al. 2016). Thus, we cannot exclude that the puberty-related increase in growth, including bone growth, had started in some of the girls in the present study. In support of this, levels of osteocalcin, as well as IGF-1, IGFBP3, and PTH were higher in the girls than in the boys.

Vitamin D is a hormone involved in calcium metabolism, regulating calcium absorption in the intestine and acting directly on bone tissue in an autocrine/paracrine way. Vitamin D deficiency leads to aberrant bone mineralization and, in severe cases, rickets in children (Morris et al. 2012). We found a robust inverse association between the children's urinary and Ery-Cd (at both 9 and 4.5 of y of age) and vitamin D3 concentrations (25-hydroxyvitamin D). For every doubling of U-Cd at 9 y of age, the mean vitamin D3 concentration decreased by −2.6 nmol/L (95% CI: −4.3, −0.83), corresponding to 0.16 SD. Studies investigating the relationship between cadmium exposure and vitamin D in children are scarce. A Mexican study of adolescents, 14 y of age, found no association of U-Cd with either 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D (active form) concentrations (Zamoiski et al. 2014). Both the median U-Cd concentration (∼0.22 μg/L) and the 25-hydroxyvitamin D concentration reported (∼60 nmol/mL) (Zamoiski et al. 2014) were quite similar to the concentrations in the Bangladeshi children at 9 y of age (0.28 μg/L and 64 nmol/L). The long-term consequences of a cadmium-related decrease in circulating vitamin D levels, which appear to be low in most Southeast Asian countries (Akhtar 2016), warrant further research.

We observed an inverse association between maternal cadmium exposure in pregnancy with the children's plasma TSH concentrations. Furthermore, childhood Ery-Cd was inversely associated with TSH in the boys, whereas no association was observed in girls ($p_{interaction} = 0.038$ and 0.18 with exposure at

4.5 and 9 y of age, respectively). Thyroid hormones are also involved in the regulation of bone growth during childhood, both within the hypothalamic-pituitary-thyroid axis, involving systemic effects, and directly on osteoblasts and osteoclasts in bone (Combs et al. 2011). However, because the concentrations of the active form free triiodothyronine (T3) and of the prohormone thyroxine (T4) were not available, we cannot draw firm conclusions on the relevance of the association with TSH. Furthermore, TSH was available for only a subsample of the children ($n = 299$). In a cross-sectional study of 1,587 adults in the U.S. National Health and Nutrition Examination Survey (NHANES), blood cadmium was found to be inversely associated with TSH (Yorita Christensen 2013), and U-Cd was found to be positively associated with both T3 and T4.

Interestingly, there were indications that increasing U-Cd concentrations during childhood were associated with decreasing plasma concentrations of IGF-1, a strong determinant of growth in children (Kanbur et al. 2005). In line with that, the association of children's U-Cd concentration with WAZ at 9 y of age was markedly attenuated by further adjustment by IGF-1. An inverse association of cadmium exposure with IGF-1 has previously been observed in male rats exposed to very high doses of cadmium in their drinking water (50 mg/L) (Turgut et al. 2005). The IGF-1 levels in boys in the present study were also lower than those in girls. The most abundant binding protein for IGF-1 in plasma is IGFBP3, which carries more than 75% of the circulating IGF-1 (Jogie-Brahim et al. 2009). Indeed, we found a fairly strong correlation between IGF-1 and IGFBP3 ($r_S = 0.48$). In spite of this, there was an indicated positive association of both maternal U-Cd and Ery-Cd with IGFBP3 in the boys and inverse in the girls ($p_{interaction} = 0.045$ and 0.15 for U-Cd and Ery-Cd, respectively).

In rural Bangladesh, children are exposed to cadmium mainly through the food. Rice, the staple food, is known to take up much cadmium (EFSA 2009). Similar exposure levels are found in other populations with a rice-based diet (Watanabe et al. 2013), implying that hundreds of millions of people are exposed lifelong to elevated cadmium levels, starting early in life (Kippler et al. 2010). In addition, we found that the children's Ery-Cd concentrations increased with decreasing iron stores (lower plasma ferritin), suggesting increased intestinal uptake through the iron transporter divalent metal transporter 1 (DMT1), as found in adults (Berglund et al. 1994). Thus, children in many low-income areas appear to be particularly at increased risk of elevated cadmium body burden.

A strength of this study is the prospective design, which is fundamental for assessing effects of exposure early in life on health consequences much later, and less likely to depend on reverse-causation. Moreover, early-life cadmium exposure was assessed by concentrations in both urine and blood: reflecting long-term exposure and ongoing exposure, respectively. Indeed, the U-Cd increased by age, whereas Ery-Cd remained rather constant over time. We find it unlikely that our observed associations of cadmium and effect biomarkers in urine would be merely a result of physiological factors as previously proposed (Wang et al. 2017) because the association between U-Cd and calcium was inverse, and urinary DPD and plasma osteocalcin were correlated.

One of the major limitations of the present study is that no measurements of bone mineral density were performed. Such measurements could have indicated whether the associations between cadmium exposure and bone-related biomarkers actually resulted in functional changes in bone health. However, such effects may not be obvious until later in life, possibly affecting peak bone mass, which is achieved between 20 and 30 y of age (Berger et al. 2010). In addition, we could measure only the children's TSH, not T4 and T3 because the blood samples were collected in sodium heparin tubes, and sodium heparin is incompatible with the analytical method used. Moreover, TSH was measured in a subsample of the children, and therefore, this data should be interpreted with caution. Similarly, analyses with Ery-Cd, especially at 4.5 y of age and prenatally, as well as the analyses stratified by gender, were restrained by the small sample sizes. Another limitation is that puberty was not assessed, and if some of the children had already entered this stage, this may have affected some of bone-related biomarkers. Finally, we cannot exclude that our findings may have been influenced by residual or unmeasured confounding.

In conclusion, we found evidence that chronic cadmium exposure during early childhood might affect bone remodeling and growth at a prepubertal age. More research in other populations is warranted to have data on the generalizability of the results. Future perspectives include assessing whether the indicated cadmium-related associations with bone biomarkers before adolescence may have long-term consequences in the form of lower peak bone mass and poor bone health, for example, osteoporosis in adulthood.

Acknowledgments

We thank all the participating families, everyone involved in the sample and data collection, and those who have been involved in the analyses of trace elements and all the different bone-related biomarkers. This work was funded by the Swedish Research Council Formas (grant 210-2013-751), the Swedish Research Council (grants 2013-2269, 2015-03206, 2015-03655), the Swedish International Development Cooperation Agency (Sida) (grants 2002-067, 2003-201A, 2015-03206), Karolinska Institutet and International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). The MINIMat supplementation trial in pregnancy was funded by Sida, Uppsala University, the UN International Children's Emergency Fund, UK Medical Research Council, Swedish Research Council, UK Department for International Development, icddr,b, Global Health Research Fund-Japan, Child Health and Nutrition Research Initiative, and U.S. Agency for International Development.

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